Effect of a Drawing Task on Cortical Excitability

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I declare that this report is my own original work and that contributions of others have been duly acknowledged.

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Table of Contents

List of tables and figures

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Abstract

The present study examined the effects of a well-practiced motor skill on measures of primary motor cortex (M1) and corticospinal activity in a small sample of eight individuals. A drawing/geometric symbol copying task served as the model for a complex overlearned motor task, commonly performed in the course of daily life. Measures of post-task M1 activity were obtained using transcranial magnetic stimulation (TMS)-evoked electromyographic measures from two intrinsic hand muscles. These were evaluated with respect to the magnitude, time-course and variability of changes that reflect modulation of M1/corticospinal excitation-inhibition. Results of the study indicated that the drawing task had minimal influence on measures of M1/corticospinal excitability or variability, up to 15 minutes post-task. The practical implication of this study finding is that routine activities of daily living involving hand muscle use, including those that are complex in nature, appear to have minimal influence on TMS measures of M1/corticospinal excitability. Therefore, the usual daily activities that individuals engage in prior to participation in TMS studies do not appear to significantly bias TMS-evoked baseline measures of M1/corticospinal activity.

Activities of daily living are invariably complex in nature, but, by virtue of being well-practised and having reached the level of procedural automaticity, are executed with fluency and without explicit cognitive effort. Extensive use of the hands is a defining feature of the majority of these activities, which include tasks such as writing, drawing, keyboard and mouse use, and the manipulation of a variety of tools and utensils. Functional task-specific use of the hands requires, for well-practised as well as unfamiliar tasks, complex, synchronised and integrated sensorimotor activity across a distributed network of brain regions, including the primary and secondary sensorimotor cortices, premotor cortex, dorsolateral prefrontal cortex, posterior parietal cortex, cerebellum, thalamic nuclei and basal ganglia (Reis et al, 2008). Whilst there is little known about the influence of motor activities performed in the course of daily life on primary motor cortex (M1) excitability, there is consensus that M1 provides the majority of cortical output to descending motor commands for planning, integration, control and execution of motor tasks, with additional roles in cognition and the early (skill acquisition) and late (consolidation) phases of motor learning (Muellbacher et al., 2002; Reis et al., 2008). M1 is thus extensively engaged in the execution of all motor activities, both well-learned and novel, but differs in quantitative and qualitative ways in this engagement (Kouchtir-Devanne, Capaday, Cassim, Derambure & Devanne, 2012; Lehericy et al., 2005; Pascual-Leone et al., 1994, 1995; Stinear & Byblow, 2003; Stinear, Coxon & Byblow, 2009; Ungerleider, Doyon & Karni, 2002). Consequently, M1, by virtue of its anatomical accessibility and central role in cortical output for motor activities, has been the focus of extensive investigation using several non-invasive imaging modalities, including transcranial magnetic stimulation (TMS).

An implicit assumption in many TMS studies is that, when called upon for motor task performance, rapid and extensive M1 engagement occurs, followed by equally rapid disengagement on task completion. The corresponding changes in indices of M1 excitation-inhibition accompanying task execution, likewise, are assumed to return to baseline and a state of readiness for subsequent activation. In human studies there is remarkably little empirical evidence directly addressing this assumption, yet the assumption informs, at a practical level, the conduct of TMS experiments such as occur in the Motor Control Laboratory in this university (and many others). Typically the majority of participants in such studies are undergraduate students who have been engaged in extensive writing/typing or other activities using their hands in the hours prior to participation in an experimental study, followed by further handwriting for informed consent, medical questionnaires and such like prior to study commencement. Consequently, activation of at least two principal intrinsic hand muscles, abductor pollicis brevis (APB) and the first dorsal interosseus (FDI), both of which commonly feature as target muscles for subsequent TMS-evoked electromyographic (EMG) measures of M1/corticospinal activity, has been present essentially right up until the start of the experimental protocol. The assumption that cessation of writing/hand use corresponds with rapid decay of M1 activity and restoration of TMS-derived measures to baseline is clearly central to proceeding with experimental protocols that subsequently evaluate the same (or adjacent) muscles. Empirical verification of this assumption has important practical implications for the conduct of TMS protocols in all applications, investigative through to therapeutic (Tanaka, Sandrini & Cohen, 2011).

TMS mechanisms and EMG measures

Cortical motor neuronal excitability changes, assessed in-vivo with animal studies (Nudo, Milliken, Jenkins, & Merzenich, 1996; Sanes & Donoghue, 2000), can be assessed non-invasively in humans using TMS, to a high degree of temporal resolution and reasonable spatial resolution (Walsh & Pascual-Leone, 2003). TMS uses a magnetic field generated from a rapidly changing electrical current in a metal coil (Barker, Jalinous & Freeston, 1985). This magnetic field can pass through the scalp, skull and meninges with minimal impedence, and induce an electrical current in the underlying cortical tissue, activating motor neurons and resulting in electrical activity descending along the neurons of the corticospinal pathway. If this electrical activity is of sufficient intensity, a 'twitch' or contraction of the corresponding (contralateral) peripheral muscle is evoked (Di Lazzaro et al., 2004). Surface EMG over the target muscle(s) enables measurement of the muscle twitch (contraction) by the motor evoked potential (MEP) recorded.

The MEP gives information about several aspects of M1 and the descending motor pathway. MEP latency, the interval between TMS stimulus delivery and the twitch response in peripheral muscle, reflects the total motor conduction time from cortex to target muscle (Badawy, Loetscher, Macdonell & Brodtmann, 2012). Motor threshold (MT) reflects the global excitability of the motor corticospinal pathway, and is defined as the minimum TMS intensity required to elicit an identifiable MEP of $\geq$50μV amplitude in ~50% of trials. MT is used for setting subsequent TMS stimulus intensities as a percentage of the resting (RMT) or active threshold (AMT) value. The small muscles of the hand have the lowest MT, reflecting the greater cortical motor representation for these muscles (Rossini, Rossini & Ferreri, 2010).

MEP amplitude reflects the sum of M1 and corticospinal pathway excitation, and is a key measure of *net* excitation in TMS studies (Hallett, 2007). Amplitude reflects the influence of a number of intrinsic and extrinsic factors. Intrinsic factors include (i) the density of cortico-motor neuronal projections onto spinal motor neurons, reflected by different muscles differing in the density of these projections, and correspondingly in MEP amplitude for a given TMS intensity (Rossini, Rossini & Ferreri, 2010), and (ii) the physiological state of M1, which is characterised by dynamic fluctuations within a physiological range, enabling maintenance of homeostasis as well as a preparedness for rapid response when called upon for diverse tasks (see Muller-Dalhaus & Ziemann, 2014, and Rossini et al., 2010 for reviews).

Extrinsic factors impacting MEP amplitude include (i) the intensity of the magnetic stimulation, and (ii) the different stimulation paradigms that manipulate the timing and frequency of the TMS stimuli delivered through the scalp, enabling varying degrees of excitation or inhibition to be evoked in the corticospinal and intracortical pathways (Kujirai, 1993). Aside from TMS-related variables, a number of other factors can impact M1 activity, to a lesser or greater extent for a given individual, setting or experimental paradigm, and contribute to changes or variability in MEP amplitudes and related measures (Orth, Snijders, & Rothwell, 2003; Ridding & Ziemann, 2010; Wasserman, 2002). An additional measure, the cortical silent period (CSP), defined as the period of EMG silence that occurs after the MEP when TMS is delivered to M1 during muscle contraction, is a measure of intracortical inhibition[1]

[1] note: several other measures of intracortical inhibition exist, however, will not be considered in detail here.

(ICI). Overall, TMS stimuli can evoke either excitation or inhibition of brain activity, with both likely occurring to a different extent and time course for each stimulus. The ensuing magnitude and pattern of responses enables localisation and measurement of brain activity in space and time (Hallett, 2007).

Motor learning

The principal focus in TMS studies in humans has been motor learning, using a range of paradigms focussed on investigating skill acquisition and motor adaptation (see Tanaka, Sandrini & Cohen, 2011 for a review). These studies have invariably used simple novel tasks that afford a high degree of experimental control, but are often lacking in ecological validity. Whilst novel task learning paradigms have shed substantial light on the mechanisms of M1 and corticospinal activity related to learning and memory (Classen, Liepert, Wise, Hallett & Cohen, 1998; Muellbacher et al., 2002; Pascual-Leone, Grafman & Hallett, 1994), there is an emerging body of evidence highlighting the substantial differences between the cortical processing and activity associated with simple compared to complex tasks (Boisgontier, Wittenberg, Fujiyama, Levin & Swinnen, 2014; Carey, Bhatt & Nagpal, 2005; Pascual-Leone et al., 1995), and novel compared to well-practised motor tasks (Boisgontier et al., 2014; Meister et al., 2005). In considering the influence of routine motor tasks on M1 excitability, these dimensions of complexity and novelty become important.

Task complexity and novelty

Simple tasks can be considered to be those comprised of simple motor responses, for example, isolated muscle contraction, repetitions of thumb abduction, thumb-index finger opposition/pinch grip, or simple reaction time type tasks. In contrast, complex

tasks can be considered to be those requiring extensive motor, somatosensory or cognitive input and integration, for example, complex digit or limb sequencing tasks, choice reaction time tasks, visual tracking and pointing tasks, or their naturalistic equivalents - writing, drawing, or playing a musical instrument. While the difference between prototypes of simple versus complex, and novel versus well-practiced tasks is clear, for many motor activities and settings these distinctions are not clear-cut. Routine activities of daily living, for instance, are well-practiced and therefore executed with fluency and ease, but are fundamentally complex skilled procedures requiring integration of multiple motor and cognitive subroutines (Carey, Bhatt & Nagpal, 2005).

There is general consensus across experimental findings that complex tasks are more effective at inducing motor cortical reorganisation (an early change indicative of brain plasticity and learning) than simple tasks. Pascual-Leone and colleagues (1995), reported increased (TMS-evoked) cortical excitability in M1 following the learning and execution of a complex novel fine motor skill (a one-handed, five-finger exercise on the piano, for 2 hour practice sessions/day for 5 days) compared to a less complex task (unstructured piano practice for the same duration/day), and concluded that the cognitively demanding aspect of the task (i.e. explicit finger sequencing) was the dimension that evoked M1 excitability rather than the purely motor dimensions of the task. Boisgontier and others (2014), using reaction time (RT) as an index of the processing complexity underlying upper and lower limb movements, found that the limb interactions mediating frequently executed "daily activities", were *complex*[2] but also highly optimised, especially for the upper limbs, and this was reflected in

[2] complexity in this study was defined as the necessity for both *recruitment* of the necessary number of limbs and *selection* of effector and non-effector muscles (i.e., coupling-decoupling) to enable execution of the task at hand

lower processing times compared to *novel* (non-functional[3]) combinations of limb recruitment and selection. As with the Pascual-Leone et al. (1995) study, the task-dependent sequencing of limb (or digit) interactions was found to be the variable that indexed complexity, with evidence of this complexity manifest as increased M1 cortical representation (plasticity) and increased choice RT measures for these studies respectively.

Simple tasks, nonetheless, do influence M1 excitability, particularly if they are novel and undertaken in the context of learning, with robust evidence of changes including increased MEP amplitudes and decreased ICI mediating task-specific muscle selection and task execution (Stinear & Byblow, 2003; Garry, Kamen & Nordstrom, 2004). In simple tasks, however, these changes are transient and generally revert to baseline within 20-30 minutes (Classen, Liepert, Wise, Hallett & Cohen, 1998; Garry et al., 2004).

Background level of motor skill training ('expertise') is a salient factor, and interacts with task demands, altering effects on M1 activity. Meister and colleagues (2005), using functional magnetic resonance imaging (fMRI), found that complex movement sequences on a keyboard showed greater fMRI activation of motor cortical areas than simple sequences in novices when compared to trained musicians. These findings, reflecting training-related adaptations (i.e., lower thresholds for induction of task-dependent excitation and inhibition, across different task types), have been replicated across a number of TMS studies (Nordstrom & Butler, 2002; Rosenkranz, Williamon, & Rothwell, 2007). Therefore, task demand (*novelty and/or complexity*)

[3] *non-functional* in the sense that these limb combinations are not routinely and ordinarily coupled for execution of daily tasks such as manipulating tools/utensils and such like

and baseline level of experience interact in shaping extent and magnitude of motor cortical activity.

M1/corticospinal activity associated with well-practised/overlearned tasks

The impact of routinely performed well-practiced motor activities on the baseline functional state of M1 activity is relatively understudied in contrast to the extensive investigations into learning and memory. What can be inferred from studies of memory mechanisms is that practice-dependent increases in MEP amplitude (M1 excitation) become attenuated once a skill has been *overlearned* (Muellbacher et al., 2001), with imaging findings showing dynamic changes in activation patterns across cortical and subcortical regions with skill acquisition, reflected in a shift away from M1 when levels of skill automaticity increase (Lehericy et al., 2005; Puttemans, Wenderoth & Swinnen, 2005). These findings, however, have emerged from studies utilising simple, novel tasks. Empirical data documenting the time-course of intra- and post-task M1 excitability changes with performance of complex and/or well-practiced naturalistic tasks are lacking.

Temporal relationship of motor activity with TMS application

Proximity of simple motor activities, such as brief duration hand muscle contraction, to subsequent TMS application is an area of active research. This relationship has been investigated in a number of recent studies, prompted in part by the observation by some research groups of antecedent hand muscle activity eliciting greater variability in measures of intracortical excitatory networks when compared to experimental cohorts with no motor pre-activity (Goldsworthy, Muller-Dalhaus, Ridding & Ziemann, 2014; Goldsworthy, Pitcher & Ridding, 2012). Other groups

have reported reversal of responses to TMS plasticity-inducing protocols (facilitation becoming inhibition, for instance) attributable to motor activity pre-stimulation (Gentner et al., 2008; Iezzi et al., 2008). These findings, however, are not consistent across the TMS literature. Sale, Ridding and Nordstrom (2007) found that the voluntary hand muscle contraction performed for the CSP measure did *not* significantly affect the extent of motor evoked potential (MEP) facilitation in an intrinsic hand muscle. Doeltgen and Ridding (2010), in examining the influence of routine activities over the course of the day on a range of TMS measures of M1 activity, found no evidence of change in motor thresholds (RMT or AMT), MEP amplitudes, intracortical inhibition or facilitation. Their study participants reported, in addition to usual activities of daily living during the day, at least 4 hours of keyboard and mouse use, as well as hand muscle contraction at the commencement of the TMS study to establish the AMT value. Thus robust engagement of M1, in a largely naturalistic manner, was undoubtedly present in the time period between measures. The precise temporal relationship of muscle activation to subsequent TMS testing, however, was unclear, so this study left unresolved the question of whether these types of motor activities, performed in close temporal proximity to TMS application, result in effects on M1 that are of sufficient magnitude and duration to alter or bias TMS-evoked baseline measures.

Study aim

Given these discrepant findings and unresolved issues, the current study sought to investigate the time-course of M1-related effects elicited by a complex, well-practiced naturalistic task performed routinely in the course of daily life. The fundamental question the study sought to answer was whether the M1/corticospinal

activation underpinning execution of a well-practised task is sustained beyond task execution.

Experimental task

A symbol drawing/copying task was selected to test our study question. In the experimental literature, drawing tasks serve principally as control/contrast conditions in studies evaluating language or higher-order cognition, with drawing considered to comprise of predominantly motor and non-linguistic elements (Horovitz, Gallea, Ali Najee-ullah & Hallett, 2013; Papathanasiou, Filipovic, Whurr, Rothwell & Jahanshahi, 2004). Whilst a cognitive processing 'load' is clearly required for execution of drawing/geometric symbol copying, empirical findings suggest that this load is of lesser magnitude (Horovitz et al., 2013), and more bilaterally distributed at a cortical network level than the strongly dominant hemisphere localised processing demands of a language-based handwriting task (Brown & Kosslyn, 1993; Harrington, Farias, Davis & Buonocore, 2007; Sturz, Edwards & Boyer, 2014). Additionally, a substantial component of automatic task execution is known to be mediated at cerebellar and subcortical (basal ganglia) levels (Lehericy et al., 2005), so in sum, the load imposed on M1 from the cognitive aspects of this task were minimised as much as feasibly possible.

The drawing/symbol copying task fulfilled the following criteria: (i) *complexity*, by virtue of the underlying dynamic interaction of the motor, cognitive and visual systems required for drawing execution, with corresponding modulation of M1 excitability as the output mechanism (Filipovic, Papathanasiou, Whurr, Rothwell & Jahanshahi, 2008), (ii) *well-practiced/highly overlearned* skill at the level of

procedural automaticity, with literate individuals having ~15 or more years experience in handwriting/drawing by adulthood (Horovitz, Gallea, Ali Najee-ullah & Hallett, 2013), (iii) a task that is engaged in *daily* (or has analogues such as typing/texting that are practised daily), (iv) a *lack of novelty*, achieved by the use of familiar symbols for the copying dimension of the task – a tick, cross and circle (✓✖○), ensuring that no new learning was required, and thus avoiding a confound-related increase in M1 activity attributable to task novelty, (v) *activation of the intrinsic hand muscles* that are the prime effectors in handwriting, FDI and APB, via the 'tripod' grip required for pen holding, (vi) task *duration* of sufficient length (several minutes) to replicate the extent of intrinsic hand muscle use that precedes a typical TMS study, and (vii) minimal *linguistic* load, due to the known effects of language (receptive or expressive) in enhancing M1 excitation (Bracco et al., 2009; Papathanasiou, Filipovic, Whurr, Rothwell & Jahanshahi, 2004; Walsh & Pascual-Leone, 2003).

Hypothesis

On the basis of findings from the available literature, it is hypothesised that a well-practiced motor task (drawing/symbol copying), due to its inherent *complexity*, will elicit robust M1 activation during task execution (i.e., increased MEP amplitudes and decreased ICI), with evidence of this activation present immediately post-task (i.e., resolution to baseline not as rapid as for a *simple* motor task). But, given that the task is *well-practised* and no new learning is occurring, these M1-related changes will not be sustained beyond the immediate post-task period, and will have returned to baseline by 15 minutes post-task.

Methods

Participants

Eight healthy right-handed participants (4 females/4 males, mean age = 31.1 years, range = 23-48 years) underwent testing for the experimental drawing task in the Motor Control Laboratory at the University of Tasmania (UTAS). All participants were volunteers, with the majority recruited from the undergraduate student population on campus. A standard medical screening questionnaire was used to exclude individuals with neurological or neuromuscular disorders or other TMS contraindications (Keel, Smith & Wasserman, 2001; Senior, 2002).

Handedness was screened for by: (1) asking subjects which hand they used for writing, and (2) completion of a handedness inventory (Oldfield, 1971). Use of a conventional 'pencil grip' (i.e., pencil held in a stable position between the thumb, index and middle fingers in a 'dynamic tripod grip') was verified in all participants given the focus on activation of the first dorsal interosseus (FDI; index finger) and abductor pollicis brevis (APB; thumb) muscles in the experimental task. Additionally, participants were questioned regarding (1) the activities they had performed in the hour prior to study participation, to screen for excessive hand-related musculoskeletal activity (for example, sustained writing/typing, musical instrument practice or other repetitive activities), (2) duration of hours awake prior to testing, (3) nicotine use, and (4) medication use, as these factors have been reported to have variable effects on cortical excitation-inhibition in the TMS literature (Chipchase et al., 2012; Ridding & Ziemann, 2010). No participants were excluded on the grounds of excessive activity, sleep deprivation, or nicotine dependence. One participant was a regular cigarette smoker, with very low level dependence as per

Fagerstrom criteria (Fagerstrom, 1978). No participants were taking prescribed psychotropic, anticonvulsant, GABA-agonist or dopaminergic drugs. One participant, post-renal transplant ~10 years earlier, was on a long-term stable regime of immunosuppressant medication which included a corticosteroid (methylprednisolone).

The experimental procedures were approved by the Social Sciences Human Research Ethics Committee at UTAS (Ethics Approval no. HOOO9261; see Appendix A1) in accordance with the Declaration of Helsinki. All subjects gave written informed consent prior to study participation.

Electromyographic recording and MEP measurement

Electromyographic (EMG) activity was recorded throughout the experiments via Ag/AgCl surface electrodes placed over the right FDI and APB muscles in a belly-tendon arrangement. The reference electrode was located over the distal process of the ipsilateral radius. The raw EMG signal was amplified (1000x) and filtered (bandwidth: 20-1000 Hz) with a CED1902 amplifier (Cambridge Electronics Design, Cambridge, UK), and sampled at 5kHz using a CED 1401 data acquisition system. Sweeps were collected from 100ms pre- to 400ms post-test pulse delivery. Data was stored on a laboratory computer for on-line visual display and subsequent off-line analysis (Signal 4.09 software, Cambridge Electronics Design). TMS-evoked parameters were measured in the target muscles in the *resting* state, defined as absent or minimal background EMG activity. Verification of target muscle relaxation was achieved by: (i) visual inspection of muscle EMG activity off-line, and (ii) measurement of the maximum EMG excursion (peak-to-peak) in the interval 55 – 5ms preceding the onset of the TMS stimulus, with root mean square (RMS)

transformed EMG amplitudes <25μV defining an acceptable level of muscle relaxation (Carson et al., 2004).

Transcranial magnetic stimulation

Participants were seated in a comfortable chair in front of a desk throughout the experimental procedure, with their right arm maintained in a relaxed position. TMS was administered using two Magstim 200[2] (Magstim Co., Whitland, UK) stimulators through a single figure-of-eight coil (70mm external wing diameter) connected to a BiStim module. The magnetic coil was positioned tangentially to the scalp of the left hemisphere (contralateral to the dominant right hand), with optimal coil location designated as the site consistently yielding the largest MEPs in the FDI or APB muscle using a moderately suprathreshold intensity. This site (the 'motor hand area') was marked on the scalp using an indelible marker to facilitate consistent coil placement throughout the experiment. Induction of posterior-to-anterior current flow was achieved by orientation of the coil handle posteriorly and laterally at approximately 30-45° from the mid-sagittal line, optimising trans-synaptic activation of the corticospinal tract.

The resting motor threshold (RMT), defined as the minimum stimulus intensity eliciting MEPs of ≥ 50μV on ≥ 3 of 5 consecutive trials in the resting target muscle, was determined by a standard protocol (Garry & Thomson, 2009). This measure formed the basis of the subsequent single-pulse (TS) and paired-pulse (CS) stimulus intensity calculations. Excitability of the corticospinal system[4] was indexed by recruitment curves (RC), depicting the relationship between MEP amplitude and TMS intensity (Ridding & Rothwell, 1997). Intracortical inhibition (ICI) was indexed via two measures: (i) the cortical silent period (CSP), defined as the period

[4] comprising the primary motor cortex (M1) and descending motor efferent pathways

of EMG silence following the MEP when TMS is administered to the motor cortex during active muscle contraction, and (ii) change (attenuation) in MEP amplitude elicited by a paired-pulse TMS protocol with an interstimulus interval (ISI) of 3ms (Kujirai et al., 1993). The paired-pulse conditioning stimulus (CS) was set at 70% RMT and paired with each of the four suprathreshold increments in test TMS intensity (CS:TS) as follows: 70:120% RMT, 70:130% RMT, 70:130sp% RMT (defined below) and 70:140% RMT.

Procedure

The experiment comprised two conditions – a writing task and a drawing task – each separated by at least one week, with participants randomised to order of task completion. Only the data from the drawing task condition will be analysed for this thesis as the writing task data is the subject of a concurrent Honours thesis. TMS-evoked EMG measures for the two target hand muscles were taken at: (i) four time points: pre-task baseline 1 (B1) and 2 (B2), and post-task at 0 (P0) and 15 (P15) minutes, and (ii) at four intensities for each time point: 120, 130, 130sp, and 140% RMT (see below), with each intensity delivered in both a single-pulse (TS) and paired-pulse (CS) mode (Figure 1). The ordering of the four intensities was randomised for each participant. A measure for CSP duration was collected during active target muscle contraction at a single intensity (130% RMT; denoted '130sp'), at each of the 4 time points. For this measure, participants were instructed to hold the pen used in the experimental drawing task using a conventional 'pencil grip', and calibrate the force/pressure exerted on the pen to approximately 10% of maximal voluntary contraction (MVC). Thus for each time point (B1, B2, P0, P15) and intensity (120, 130, 130sp, 140% RMT), a total of 10 single and 10 paired TMS pulses were administered in a random sequence at a frequency of 0.2Hz controlled

by customised software. The recruitment curves (RC; test intensity versus MEP amplitude) generated for each muscle at each of the 4 time points therefore represented the 10 MEPs from each TMS mode and intensity.

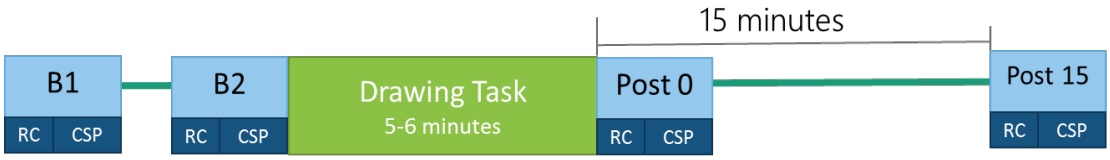

Figure 1. Timeline of the experimental protocol for pre- and post-task measurement of TMS-evoked EMG indices. Motor cortex excitation and $GABA_A$-mediated intracortical inhibition are captured in the recruitment curves, and $GABA_B$-mediated inhibition in the CSP measure.

Experimental Task

Following the baseline measures, the pen-and-paper (drawing) task was completed. Participants were instructed to copy a sequence of 3 simple geometric symbols (○✖✓) onto a grid of 200 squares on a sheet of A4 paper, resulting in a total of 600 symbols being copied. This matched the number of letters participants wrote in the writing task. Participants were encouraged to complete the task using a relaxed non-fatiguing handwriting speed. The task took 5-6 minutes to complete.

Design

This study employed a 2 x 4 x 4 repeated measures design, with *TMS type* (TS and CS), *time* (B1, B2, P0, P15) and *intensity* (120, 130, 140, 130sp% RMT) as the within-subject factors. The dependent variables were MEP amplitude and ICI across all conditions, and CSP during active muscle contraction. The CSP data was subsequently found to be unusable therefore will not be considered further.

Data Analysis

Individual MEP peak-to-peak amplitude was measured in the interval 15-60ms post-TMS stimulus, as per standard protocols (Garry & Thomson, 2009). Mean peak-to-peak amplitude of single-pulse (TS) and paired-pulse (CS) MEPs for each target muscle was calculated for each participant at each level of *time* and *intensity*.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 21 (IBM SPSS, Armonk, NY, USA). Prior to analysis, data were visually inspected for normality. For each muscle, three-way repeated measures ANOVA was used for analysis of EMG_{RMS}(background) and post-stimulus MEP amplitudes, with *TMS type* (2 levels), *time* (4 levels) and *intensity* (4 levels) as the within-subject factors. Inclusion of the '130sp' data set as a level of *intensity* enabled examination of the effect of muscle activation on M1 excitability. The Greenhouse-Geisser epsilon correction was used to adjust for violations of sphericity. Where indicated by a significant F, post-hoc pairwise comparisons were conducted with Bonferroni correction for multiple means to control for type I errors. Results were considered significant at $p<0.05$ for all analyses. All values (MEP amplitude, EMG_{RMS} amplitude) are expressed as mean ± standard error (SE), with 95% confidence intervals (CI).

Results

Analyses were performed and will be reported for both hand muscles. The experimental findings were essentially mirrored in both muscles, but in instances where discrepant findings occurred, FDI will be considered the primary muscle for interpretation of findings, as this was the muscle for which all TMS parameters were set.

1. Background EMG activity

Intrinsic hand muscle relaxation in the resting muscle conditions (120, 130, and 140% RMT) and muscle contraction in the 10% MVC condition (130sp% RMT) was verified through analysis of the EMG_{RMS} data in the interval 55-5 ms pre-TMS stimulus onset.

Background *resting* EMG_{RMS} activity was consistently less than 0.025mV (see Figure 2), the threshold generally accepted as indicating an absence of muscle activation (Carson et al., 2004). This finding was confirmed through three-way repeated measures ANOVAs for both muscles, with no significant differences in extent of background EMG activity across levels of *TMS type* or *time*:

FDI: $F_{\text{TMStype}}(1,7) = 1.72$, $p = .231$, $\eta_p^2 = .20$

FDI: $F_{\text{time}}(1.93, 13.49) = .65$, $p = .532$, $\eta_p^2 = .09$

APB: $F_{\text{TMStype}}(1,7) = .66$, $p = .443$, $\eta_p^2 = .09$

APB: $F_{\text{time}}(1.53,10.71) = .22$, $p = .746$, $\eta_p^2 = .03$

For EMG_{RMS} activity across the levels of *intensity*, a significant difference was expected between the intensities requiring muscle relaxation (120, 130, 140% RMT) compared to the intensity requiring muscle contraction (130sp% RMT). This was

confirmed with ANOVA for *FDI*: $F_{intensity}(1.02,7.14) = 15.13$, $p = .006$, $\eta_p^2 = .68$, and reached near significance for *APB*: $F_{intensity}(1.02,7.13) = 4.68$, $p = .066$, $\eta_p^2 = .40$, with higher background MEP amplitude means for both muscles for the 130sp level (see Appendix C2, Table 1.2 for means). These results indicated that participants performed the muscle contraction task as instructed, and validated the data set for the subsequent analyses.

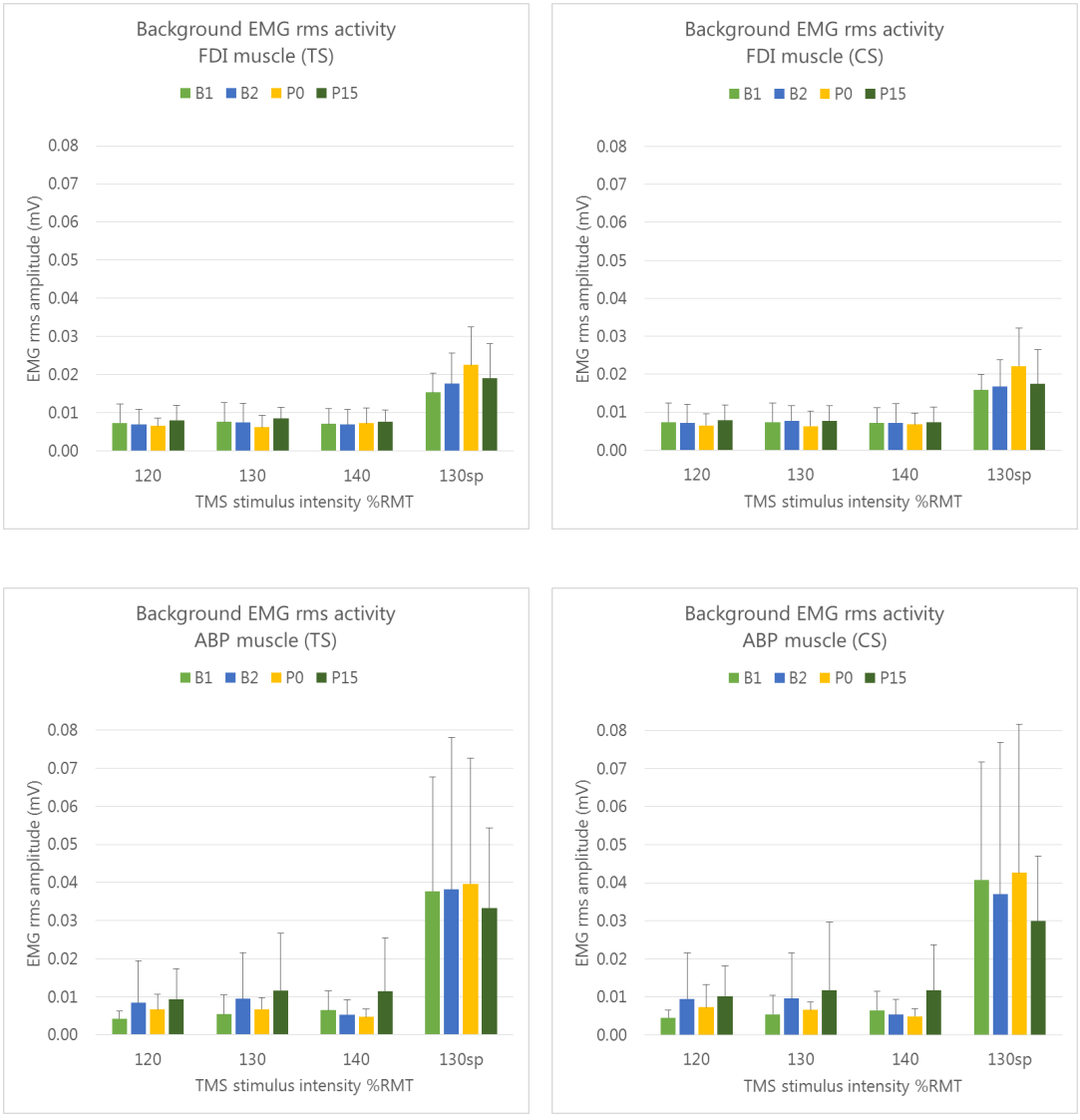

Figure **2**. Background EMG_{RMS} activity (in the 55-5ms interval pre-TMS stimulus onset) for FDI (upper panels) and APB (lower panels) muscles, across intensities (120, 130, 140, and 130sp% RMT) at each time point (B1: $baseline_1$, B2: $baseline_2$,

P0: post-task$_{0min}$, and P15: post-task$_{15min}$) for single-pulse (TS) and paired-pulse (CS) TMS type. Across both muscles, stimulus types, time points and intensities (excepting 130sp% RMT) the EMG_{RMS} activity was < 0.025mV. The error bars indicate the upper limit of the 95% CI.

2. Corticospinal excitability

2.1. Recruitment curves

MEP amplitudes from the FDI and APB muscles were obtained over a range of TMS intensities to generate recruitment curves[5] at each level of *time*. The changes in the height of each bar reflect the effect of TMS intensity (and muscle activity in the 130sp condition) on MEP amplitude. These changes are compared in Figure 3, for each *TMS type* and hand muscle, with the four levels of *time* each represented by a different bar. The means, standard error (SE) and confidence intervals (CI) for both muscles can be found in Appendix C2, Table 1.2.

2.2. FDI muscle

As expected, a clear relationship between MEP amplitude and stimulus intensity was evident across all levels of time, with the largest MEP amplitudes elicited in the '130sp' condition where the target muscle was actively contracted[6]. The effect of *intensity* was confirmed with three-way repeated measures ANOVA, with a significant main effect of intensity, $F_{intensity}(2.1,14.6) = 23.20$, $p < .001$, $\eta_p^2 = .77$. Contrary to our hypothesised prediction ($MEP_{B1} = MEP_{B2} = MEP_{Post15} < MEP_{Post0}$), no significant difference was evident in the MEP amplitude means across *time* (see Figure 3), indicated by the similarity in the height of the bars for each level of *time* at

[5] depicted as bar graphs
[6] with muscle contraction, the increased MEP amplitude is comprised of muscle contraction-related excitation of M1 and the spinal cord (Di Lazzaro et al., 2004).

each level of intensity for the respective TMS types. This lack of significant change was confirmed using a three-way repeated measures ANOVA, with a non-significant effect of *time*, $F_{time}(2.4,16.9) = .74$, $p = .514$, $\eta_p^2 = .096$.

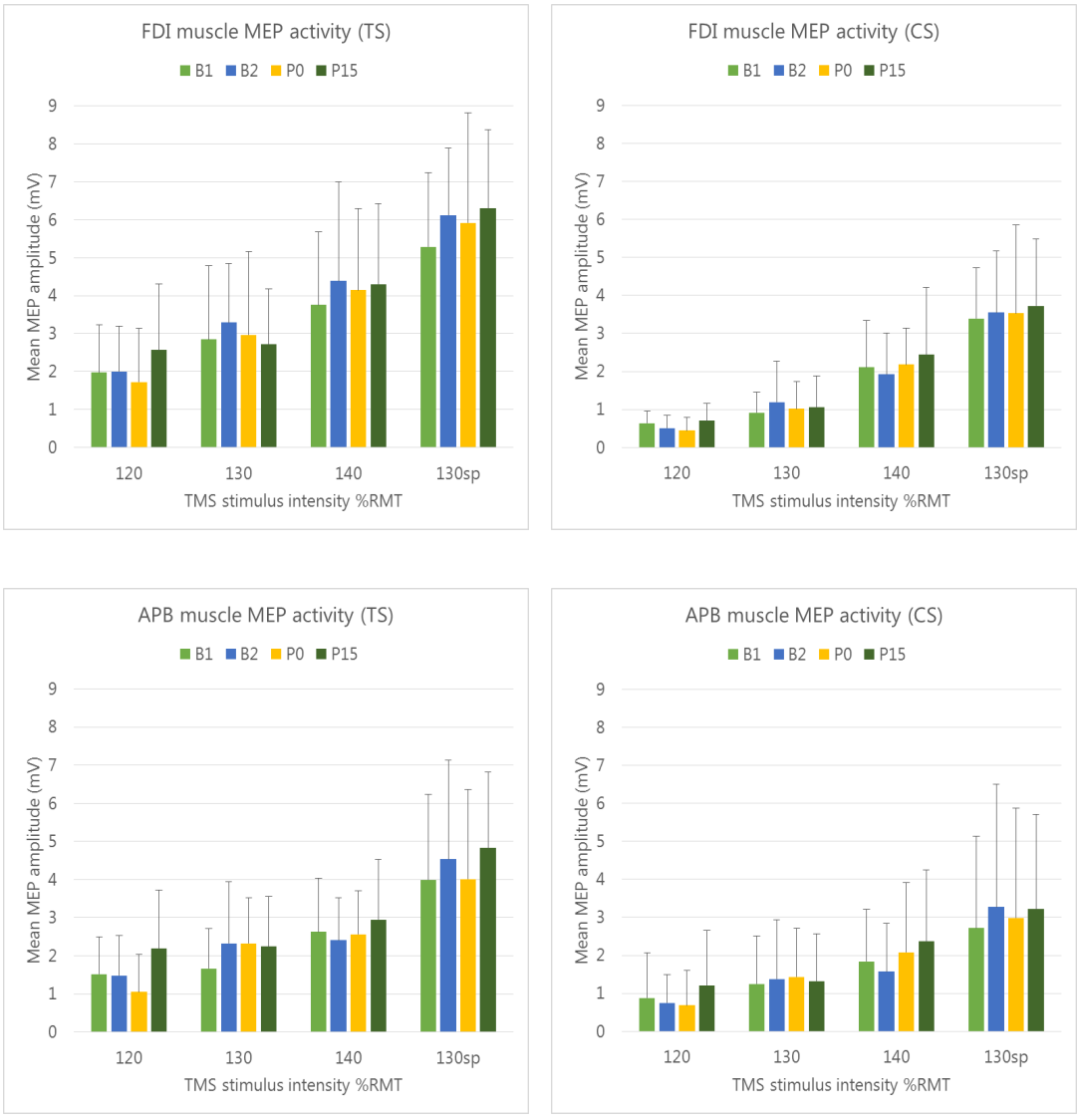

Figure **3**. EMG_{MEP} recruitment curves for each level of time (B1, B2, Post0, Post15) for the FDI (upper panel) and APB (lower panel) muscles, for single-pulse (TS) and paired-pulse (CS) TMS types. The error bars indicate the upper limit of the 95% CI. Means, SE and CIs are reported in Appendix C2, Table 1.3.

2.3. APB muscle

As for FDI, a significant main effect of intensity was present, $F_{\text{intensity}}(1.3,9.2) = 8.58$, $p = .013$, $\eta_p^2 = .55$. Again, there was no significant main effect of time, $F_{\text{time}}(2.1,14.8) = 3.33$, $p = .062$, $\eta_p^2 = .32$.

3. Intracortical inhibition

Intracortical inhibition (ICI) was elicited by paired-pulse TMS and indexed by the difference in amplitudes between the conditioned MEPs (MEP_{CS}) and unconditioned MEPs (MEP_{TS}), across time and intensity. As the main experimental concern with respect to inhibition was change in magnitude of inhibition across *time* (pre- versus post-task), the primary analysis was focussed on ascertaining a main effect of *TMS type* ($MEP_{TS} > MEP_{CS}$ means), and the presence of any *TMS type x time* interaction.

3.1. Primary analysis

3.1.1. FDI muscle: A significant main effect of *TMS type*, $F(1,7) = 8.39$, $p = .023$, $\eta_p^2 = .55$ indicated that MEP_{CS} amplitudes were attenuated relative to MEP_{TS}, consistent with activation of inhibitory circuits by the conditioning TMS stimulus (see Appendix C2, Table 1.1 for means). All second-order interaction effects involving *TMS type* were non-significant: *TMS type* x *time*: $F(1.44, 10.09) = 1.95$, $p = .194$, $\eta_p^2 = .218$; and *TMS type* x *intensity*: $F(2.17, 15.22) = 1.59$, $p = .236$, $\eta_p^2 = .185$, indicating that the extent of inhibition in this muscle did not vary significantly across the conditions of time and intensity. As per Figure 4, the bar graphs depicting FDI_{TS} and FDI_{CS} activity[7] show that the pattern of MEP activity for conditioned versus unconditioned stimuli is essentially the same for each level of time, indicating a similar magnitude of inhibition at all intensities and at each level of time.

[7] i.e., recruitment curves

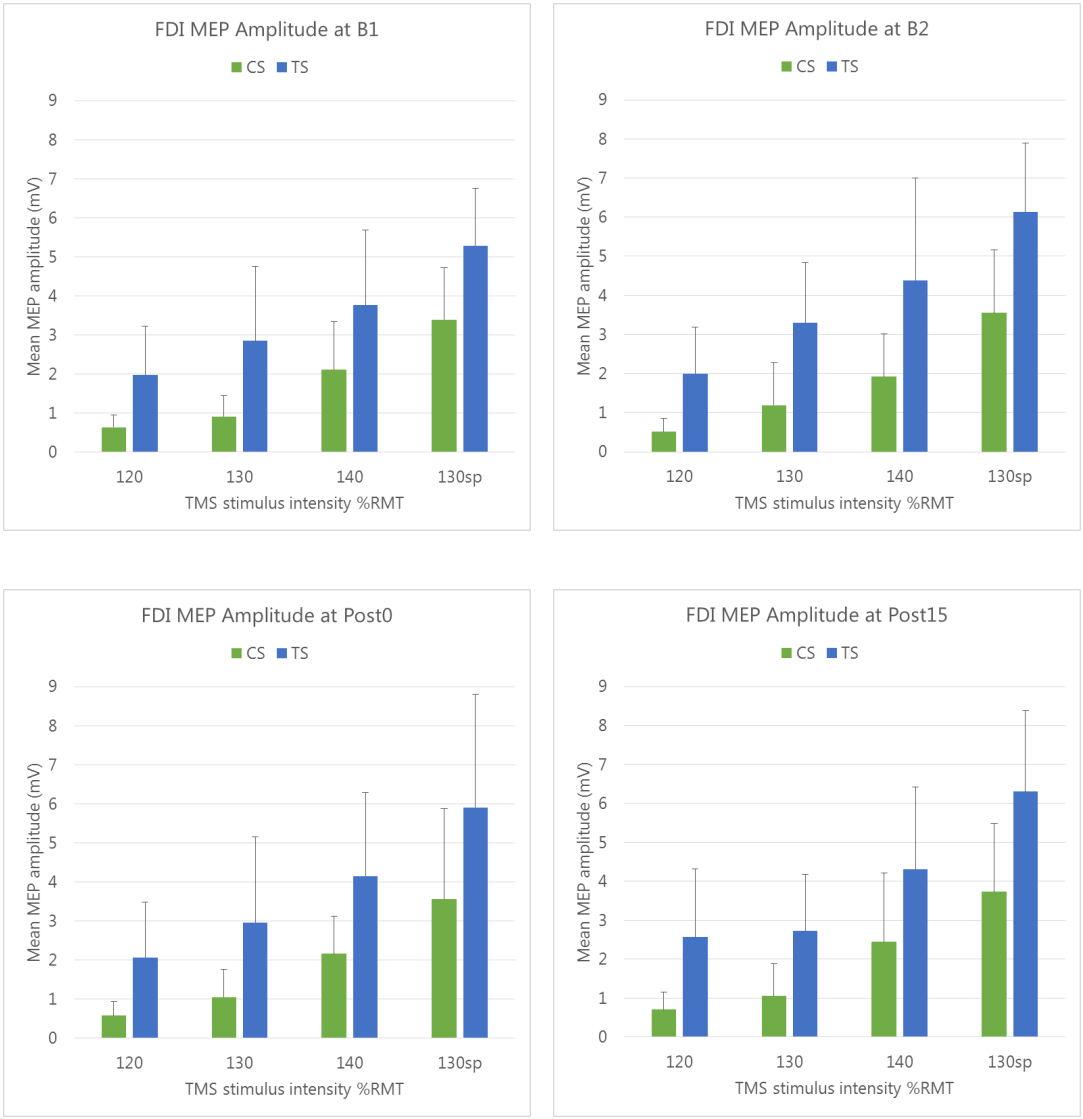

Figure **4**. FDI recruitment curves reflecting the magnitude and pattern of intracortical inhibition elicited by the conditioned TMS stimulus (MEP_{CS}) compared to the unconditioned stimulus (MEP_{TS}), across four levels of intensity, and at four time points (pre-task upper panels, post-task lower panels). The error bars indicate the upper limit of the 95% CI.

3.1.2. APB muscle: As for FDI, attenuation of MEP size was observed in the CS condition, and confirmed with ANOVA with a significant main effect of *TMS type*, $F(1,7) = 10.42$, $p = .015$, $\eta_p^2 = .60$ (see Appendix C2, Table 1.1 for means). With

respect to second order effects, the *TMS type* x *intensity* interaction was non-significant ($F(1.37, 9.55) = 3.53$, $p = .083$, $\eta_p^2 = .34$), however the *TMS type* x *time* interaction was found to be significant, $F(1.85, 12.92) = 4.15$, $p = .043$, $\eta_p^2 = .37$ (see Table 1 for means, SE and CI). The nature of this interaction was clarified through follow-up simple effects ANOVAs of the effect of *time* for each *TMS type* (TS vs CS), via two-way repeated measures ANOVA. A significant main effect of *time* was found for TMS_{TS}: F_{TS} (2.08, 14.57) = 4.80, $p = .024$, $\eta_p^2 = .37$, but not for TMS_{CS}: $F_{CS}(1.94, 13.57) = 1.77$, $p = .207$, $\eta_p^2 = .20$, indicating that the *TMS type* x *time* interaction was produced by changes elicited by the unconditioned TMS stimulus (TS; indexing excitation), and not by the conditioned TMS stimulus (CS; indexing inhibition).

3.2. Secondary analysis

Effect of time on MEP_{APB_TS}: As per Table 1 (below), the MEP_{TS} amplitude means for APB muscle varied in magnitude at both pre-task and post-task time points. To clarify the significant main effect of *time* for TMS_{APB_TS}, post-hoc pairwise comparisons (with Bonferroni correction for multiple means) were performed for all the levels of time. These comparisons, however showed no significant differences between or within the pre-task and post-task time points (see Appendix C2, Table 2 for pairwise comparisons).

Table **1**.

APB muscle: Effect of *time* on *single-pulse (TS)* (upper panel) and *paired-pulse (CS)* (lower panel) TMS-elicited MEP mean amplitudes (mV), ± SE, and CI. A significant main effect of *time* was found for TMS_{TS} but not for TMS_{CS}.

Muscle (TMS type)	Time	MEP Mean (mV)	Std. Error	95% Confidence Interval
APB (TS)	B1	2.450	0.553	1.142 - 3.758
	B2	2.687	0.583	1.308 - 4.067
	Post0	2.489	0.516	1.270 - 3.709
	Post15	3.053	0.608	1.616 - 4.491
APB (CS)	B1	1.671	0.265	0.207 - 3.135
	B2	1.745	0.358	0.175 - 3.315
	Post0	1.795	0.329	0.187 - 3.403
	Post15	2.030	0.440	0.376 - 3.684

Given the discrepant results for FDI and APB muscles for the *TMS type x time* interaction analysis, the FDI analysis will take precedence due to it being the target muscle. On this basis, given the non-significant *TMS type x time* interaction for FDI, it was concluded that inhibition was unchanged across the levels of time (see Appendix C2, Table 1.4 for FDI means, SE and CI).

As depicted in Figure **5**, the pattern in MEP activity across *time* for each TMS type is essentially the same for each muscle.

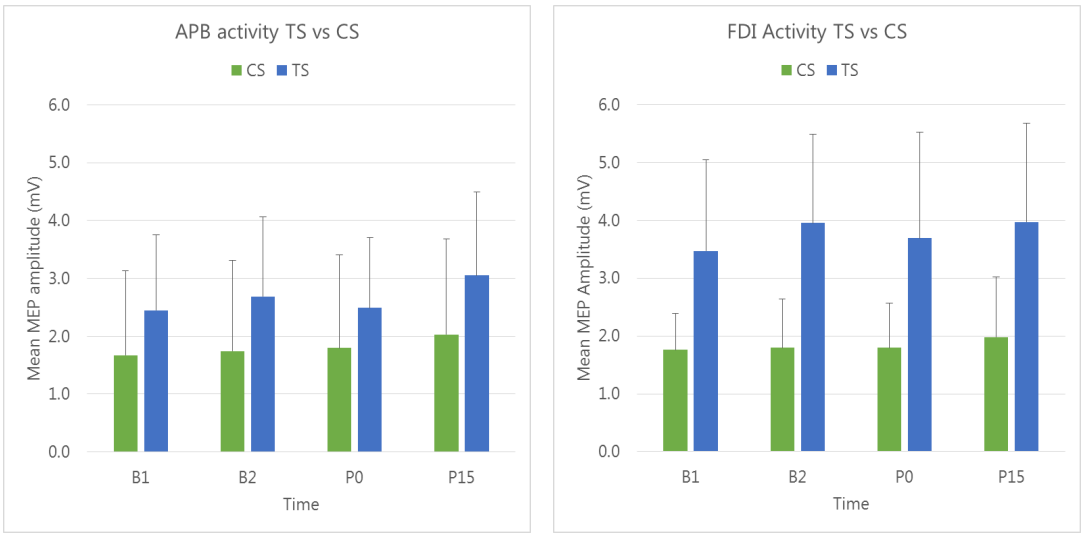

Figure **5**. MEP amplitude change across *time*, for TS and CS *TMS types*, for both FDI (left panel) and APB (right panel) muscles. The error bars indicate the upper limit of the 95% CI.

Discussion

The primary aim of the present study was to investigate the effects of a simple task of daily living on motor cortex excitability. The drawing task served as our model of an overlearned motor skill routinely performed as an activity of daily living. A secondary aim was to consider whether any effects on M1 excitability of this task might account for some of the variability that characterises particular TMS measures. Primary motor cortex (M1) and corticospinal activity pre- and post-task were indexed via MEPs elicited by TMS.

Corticospinal excitability is reliably reflected by rapid changes in magnitude and direction of MEP amplitudes at time of measurement. It was hypothesised that a routine over-learned motor activity such as handwriting (in the form of a drawing task) would have a transient effect on measures of motor corticospinal excitability and intracortical inhibition (ICI), with increased MEP amplitudes and decreased ICI evident in the immediate post-task period, followed by a return of these measures to

baseline levels by 15 minutes post-task completion. The results of the study, however, did not support the hypothesis.

With respect to corticospinal excitation, the main effect of time was non-significant for the target FDI muscle, with the MEPs demonstrating *no* significant change in amplitude between the pre-task and post-task levels. Rapid and complex integrative responses involving recruitment and disengagement of effector and non-effector muscles (in the order of milliseconds or less) are a characteristic of corticospinal system activity and require intra-task measurement to quantify. Our post-task time point of measurement was not designed to capture any intra-task excitation given the study question was focussed on post-task changes. Our findings clearly indicated that if there were any immediate post-task excitability changes, they were not sustained.

Task-dependent changes in M1/corticospinal excitation-inhibition

Modulation of M1/corticospinal excitability has been shown to be task-dependent, and influenced by the dimensions of task complexity and novelty (Carey, Bhatt & Nagpal, 2005). Complex and/or novel task execution has been demonstrated to elicit more extensive M1 activation than simple or overlearned tasks (Boisgontier, Wittenberg, Fujiyama, Levin, & Swinnen, 2014; Kouchtir-Devanne, Capaday, Derambure & Devanne, 2012; Pascual-Leone et al., 1995). M1 activity elicited by complex but clearly overlearned tasks is less clear. Our hypothesised increase of immediate post-task corticospinal excitability and decreased inhibition was derived from experimental findings demonstrating selective short-term induction of M1 excitation in response to relatively brief duration motor activity associated with novel task execution. These studies all reported effects (behavioural and/or electrophysiological) on M1 that outlasted the task duration for variable periods of

time (typically less than 20 minutes) before returning to baseline (Classen et al., 1998; Garry, Kamen & Nordstrom 2004; Muellbacher et al., 2002). The hypothesised decrease in ICI derived from findings indicating that inhibition mediates hand muscle selectivity and specificity for the pending task (Liepert, Classen, Cohen & Hallett, 1998; Stinear & Byblow, 2003; Stinear, Coxon & Byblow, 2009). These findings, however, were from *novel* task settings, and are potentially not generalisable to the overlearned task setting. Given that CS-elicited inhibition remained *unchanged* across time in the present study, there was insufficient evidence to support the hypothesis relating to intracortical inhibition. Overall, drawing task complexity did not elicit sufficient M1/corticospinal activity to be reflected in measurable sustained changes of excitation or inhibition immediately post-task.

Influence of muscle preactivity

The temporal relationship between low-grade hand muscle contraction and its potential influence on TMS-elicited measures of M1 excitability was also evaluated from our data. The phenomenon of variability in cortical measures or responses, attributed to muscle activity pre-TMS application, has been reported by some (Gentner, Wankerl, Reinsberger, Zeller & Classen, 2007; Goldsworthy, Muller-Dalhaus, Ridding & Ziemann, 2014; Goldsworthy, Pitcher & Ridding, 2012; Huang, Rothwell, Edwards & Chen, 2008[8]; Iezzi et al., 2008), but not all investigators (Sale, Ridding & Nordstrom, 2007; Doeltgen & Ridding, 2010). The mechanisms responsible for this effect elicited by a preceding voluntary muscle contraction are unclear, but intriguing, given that hand muscle contraction is procedurally *simple* and

[8] the protocol investigated was continuous theta burst stimulation (cTBS), a well-characterised technique for inducing neuroplastic changes in M1 (Huang et al., 2008)

automated, therefore theoretically the least likely task to elicit substantial effect/influence on M1 indices of excitation-inhibition.

Muscle contraction of this extent/duration is remarkably common, often performed below the level of conscious awareness (for example, as involuntary hand muscle contraction evoked by mild anticipatory anxiety) or integral to activities performed prior to TMS study participation (for example, handwriting, carrying a briefcase, gripping a car steering wheel or bicycle handles). Our drawing task, completed in approximately 6 minutes, and requiring low-grade contraction (~10% MVC) of intrinsic hand muscles for pencil grip, exceeded the duration of muscle contraction eliciting the effects on variability reported in the literature. There was no evidence of *increased* variability across the MEP amplitude means at either of the post-task time points in our data. This finding of minimal/insignificant change with respect to cortico-motor activity post-drawing does not, however, definitively exclude *indirect* or *remote* effects of the preceding motor activity on later M1 activity/response (Muller-Dalhaus & Ziemann, 2014); these types of effects, however, were not measured in this study. The potential impact of antecedent muscle activity on plasticity-inducing protocols, whether in the form of learning paradigms (with behavioural outcome measures) or TMS-based investigations or interventions is, however, of increasing practical and clinical relevance given the prevalence and breadth of TMS applications.

M1/corticospinal excitability changes associated with handwriting/drawing

Naturalistic tasks performed in the course of daily living (for example, communication via typing, email, texting, handwriting, or symbolic drawing) have cognitive as well as a motor performance dimensions, thus are both *complex* and

automated when skill is proficient (Plamondon, O'Reilly, Rémi & Duval, 2013). Due to difficulties in controlling for the varied dimensions of these types of tasks they have had less empirical study than simpler tasks. Of the imaging studies evaluating writing or reading, the majority have measured within-task changes in excitation-inhibition, principally to gain understanding of M1 mechanisms of task differentiation and execution. Therefore, empirical evidence with which to directly compare our study findings is severely limited.

Two studies (Filipovic, Papathanasiou, Whurr, Rothwell & Jahanshahi, 2008; Papathanasiou, Filipovic, Whurr, Rothwell & Jahanshahi, 2004), evaluating the effects of handwriting and low-level cognitive tasks on M1 excitation-inhibition, reported findings relevant to the outcomes of the present study. An important caveat in consideration of these findings is that of *state-dependence*, i.e., the change in the nature and dynamics of physiological interactions in different behavioural state settings, for instance, resting muscle versus active task execution states (Reis et al., 2008). These studies evaluated intra-task changes compared to our pre- and post-task time points, so extrapolation of conclusions requires caution. These studies, however, represent the few conducted with naturalistic tasks, so will be discussed from that perspective.

Filipovic and colleagues (2008) compared writing and drawing tasks on measures of M1 excitation-inhibition *during* task execution. They found a comparable level of increased corticospinal excitatory activity (indexed by MEP amplitudes) mediating the motor aspects of task execution across the three conditions (drawing, writing and a control/pen-squeezing task) of their study, but no significant additional change in measures of excitation attributable to the differing linguistic loads of the three

conditions. A change in cortical silent period duration[9] was reported for the writing task alone, and interpreted as mediating task-execution for the task with greatest linguistic 'load' (the writing task) compared to the tasks with minimal or no linguistic load (the drawing and control tasks). The focus of their study, however was measurement of indices of task differentiation, so the presence of post-task excitation-inhibition was not measured. Notwithstanding this, their finding of no significant additional or differential change in corticospinal excitability during performance of a well-practiced procedural skill (writing and drawing) is generally consistent with our finding of minimal impact of a well-practised task on corticospinal excitability.

In contrast, Papathanasiou and colleagues (2004), in a study seeking to separate out the motor and cognitive components contributing to M1 excitation in tasks such as writing and drawing, compared M1 activity from both hemispheres across a range of *non-motor* cognitive tasks. The tasks were designed to correspond with the 'low-level' cognitive elements underpinning reading and writing – both well practiced activities performed on a daily basis for literate individuals. A visual search-match paradigm was utilised with linguistic, numerical and geometric symbols as the different conditions. They found that MEP amplitudes from the right (dominant hand) FDI muscle were elevated across all tasks, with a significant difference between each task when individually compared to a control condition involving no cognitive activity. In comparisons between the tasks, no significant difference was found, however, between the linguistic and geometric symbol conditions. That study highlighted the ready facilitation of M1 by non-motor tasks, for both the *well-practised* aspects (discrimination between letters and geometric shapes) as well as

[9] a measure of $GABA_B$-mediated intracortical inhibition

the *novel* aspects (visual search-match), as well as the difficulties of evaluating novel versus well-practised dimensions of tasks and their respective contributions to cortical excitability responses. Compared to the finding by Filipovic et al. (2008), the finding of no inter-task difference on MEP amplitude measures was the same. Filipovic and colleagues, however, measured ICI in addition to M1 excitability, and it was only in this dimension that significant differences were found and attributed to the feature ('linguistic load') that distinguished the tasks. The absence of a measure of inhibition potentially limited the likelihood of Papathanasiou and colleagues detecting any task difference.

Notwithstanding some of the similarities discussed, drawing tasks do differ from writing tasks in important ways. Whilst both are skilled fine-motor tasks sharing visuo-spatial and symbolic representational dimensions, there are well established differences relating to cortical representation, with drawing more widely distributed and bilaterally represented in contrast to the strongly lateralised language processing regions in the dominant hemisphere (Bracco et al., 2009; Brown & Kosslyn, 1993; Sturz, Edwards & Boyer 2014). This clear delineation has been confirmed with functional neuroimaging, however different studies (and imaging modalities) have identified differing cortical areas (primary motor cortex versus non-motor cortices) as mediating significant drawing-writing differentiation (see Harrington, Farias, Davis & Buonocore, 2007 for a review). Extrapolating from the findings of non-TMS neuroimaging (given the paucity of TMS studies examining drawing), it is not surprising that drawing may not elicit a substantial intra-task (and corresponding post-task) response from M1 other than the background corticospinal drive required for the motor aspects of task execution, given its widely and bilaterally distributed

encoding. More studies are needed to clarify, both intra- and post-task, the nature and extent of M1 activity associated with drawing.

Cortical representational shift in early and late stage learning

In line with the discussion regarding cortical representation of drawing is the phenomenon of shift in memory encoding as tasks become well consolidated and stored in long-term memory. M1 has a critical role in early motor (Classen et al., 1998; Muellbacher et al., 2002) and non-motor learning (Sanes & Donoghue, 2000), however a shift occurs to more widely distributed network of cortical regions when a task is well-learned (Squire & Wixted, 2011; Walsh & Pascual-Leone, 2003). Correspondingly M1 becomes relatively less important, and theoretically less responsive with respect to excitation-inhibition when called upon to perform tasks that have reached the level of procedural automaticity (Rosenkranz, Kacar & Rothwell, 2007). Whilst some evidence for this shift has been demonstrated using simplified experimental tasks and other imaging modalities (Puttemans, Wenderoth & Swinnen, 2005; Ungerleider, Doyon & Karni, 2002), there is little empirical evidence from well-learned complex procedural tasks such as drawing. Nonetheless, this anatomical shift underpinning distributed storage in long-term memory likely contributed, in part, to the absence of sustained M1/corticospinal activity following the drawing task.

M1/corticospinal excitability changes associated with activities of daily living

The influence of *time of day,* whether attributable to circadian neuroendocrine effects, or other incompletely understood mechanisms that up- or down-regulate the capacity for plasticity-induction, has important practical implications with respect to the timing of investigative and therapeutic applications of TMS. Two studies have

evaluated this factor (Doeltgen & Ridding, 2010; Koski, Schrader, Wu & Stern, 2005), in largely naturalistic settings, with the aim of identifying underlying biological or other variables (e.g., muscle activity) that systematically bias measures of M1 activity. Koski and colleagues evaluated serial changes in RMT and CSP (obtained from FDI and APB) over seven TMS sessions, from both hemispheres, in a ten hour period in the same subjects. They chose not to control for variables such as test session time of commencement, fatigue, food or caffeine intake, or hand muscle use in writing/typing, on the basis that in a clinical setting these factors are largely outside the clinician's control. Hand muscle contraction preceded each CSP measure, as per standard protocol. Their findings confirmed the relative stability of MT and CSP in the group as a whole over the 10 hour period, with these measures robust to the influence of activities of daily living and hand muscle pre-activity. Doeltgen & Ridding (2010) examined *time of day* influence across a number of TMS indices and likewise found no significant effects in their participants (see earlier discussion in Introduction). Their findings highlighted the minimal influence, on a broad range of TMS measures, of cumulative hand muscle activity over the course of a day.

The ecological validity of both studies enables practical application of the finding that *time of day* (at least between the hours tested) does not appear to bias the values acquired over a wide range of TMS measures. Drawing, like the majority of activities of daily living, involves significant, dexterous and task-dependent recruitment of the hand muscles and corresponding engagement of the hand area of M1. The drawing task of our study has findings in accordance with Doeltgen & Ridding[10], with the practical implication that hand use, to the extent required by drawing, does not

[10] the CSP duration measure from our study was unobtainable, so a comparison with the Koski et al. study was not possible; in general, the stability of their group-level measures were consistent with our findings across different measures of excitation-inhibition

significantly influence the peripheral TMS-derived measures of M1 excitation-inhibition obtained from the intrinsic hand muscles.

Variability of TMS measures and contributory factors

Variability of TMS measures informed our research question, and was evident in our data, but most importantly, was not substantially increased post-drawing task. There is general consensus that resting motor threshold (Koski, Schrader, Wu & Stern, 2005) and MEP onset latency (Kiers, Cros, Chiappa, & Fang, 1993) are least affected by variability, with MEP amplitudes the most extensively impacted, with a high degree of intertrial, inter- and intra-individual variability (Boroojerdi et al., 2000; Darling, Wolf & Butler, 2006; Wasserman, 2002). Intrinsic physiological fluctuations in excitability at cortical, subcortical and spinal segmental levels are considered to underlie variability, and comprise the 'normal' dynamic state of central and peripheral nervous system activity (Ridding & Ziemann, 2010; Wasserman, 2002). Standardised protocols were adopted to minimise variability attributable to methodological factors (as per Boroojerdi, 2000; Kamen, 2004; Moller, Arai, Lucke & Ziemann, 2009; Schmidt et al., 2009; & Wasserman, 2002). Variability attributable to use of a hand-held TMS coil was likely to have been minimal. Whilst alterations in the position of a hand held TMS coil on the scalp are known to result in variations in MEP responses, the use of external apparatus (e.g., navigational systems or stereotactic frames) to clamp/stabilise the coil relative to head position and optimise stimulation site localisation has not been shown to significantly decrease MEP variability (Jung et al., 2010; Schmidt et al., 2009).

Other factors potentially contributing to variability include circadian effects on cortical excitation-inhibition (Lang et al, 2011). High cortisol levels are theorised to inhibit plasticity and learning, with plasticity effects (MEP facilitation) reported to be

increased in the afternoon when endogenous cortisol levels are low (Sale, Ridding & Nordstrom, 2007, 2008).

Our experimental testing was performed in the morning and early afternoon (9am to 4pm). Plasma cortisol levels are maximal in the morning immediately post-waking, decrease rapidly in the initial 1-2 hours, and further decrease to a trough level 14 hours post-waking (Ranjit, Young, Raghunathan & Kaplan, 2005). Seven of our eight participants had been awake for two or more hours prior to testing, and all eight were tested within 10 hours of waking, thus any cortisol-mediated circadian effects were minimised. The sole participant who had been awake less than one hour was also the sole cigarette smoker. Nicotine has been reported to have variable effects on TMS-elicited indices of excitation-inhibition (Lang, Hasan, Sueske, Paulus & Nitsche, 2008). For this participant, the data collected did not represent extremes of the MEP amplitude range, so the influence and/or interaction between a diurnal cortisol peak and nicotine-related effects on the measures collected are unlikely. An additional participant was on a long-term post-renal transplant regime of immunosuppressant and corticosteroid drugs. The testing for this individual occurred ~8 hours post-corticosteroid ingestion, was therefore remote from any pharmacologically-derived cortisol 'peak', and elicited measures in the mid-range of the group values.

Motor cortex and corticospinal activation, independent of muscle activity, are also responsive to multiple influences including attention (Kiers, Cros, Chiappa, & Fang, 1993; Rosenkranz & Rothwell, 2004; Thomson, Garry & Summers, 2008), varying sensory inputs (Rosenkranz & Rothwell, 2004, 2012), mental imagery (Alaets et al., 2010; Fadiga, Fogassi, Parezi & Rizzolati, 1995; Strafella & Paus, 2000) and

movement preparation (van Elswijk, 2007). These factors variably influence excitability of the motor corticospinal pathway and subsequent responses to TMS stimuli. Some of these factors may operate below the level of conscious awareness, so experimental controls to minimise their influence vary in efficacy.

Notwithstanding attention to extraneous and methodological variables, MEP amplitude variability was still evident in our data, to an extent that attained statistical significance for one of the intrinsic hand muscles (APB, the non-principal target, for TMS_{TS} mode alone).

Study limitations

The subjective calibration of force production in the muscle activation condition (10% MVC, 130sp% RMT) was an intentional decision. In clinical settings there is substantial reliance on an individual's subjective report/perception of force exertion as direct measurements are not always available to objectively quantify the magnitude of forces being exerted (Koski, Schrader, Wu & Stern, 2005). Whilst objective verification of the accuracy of force calibration is desirable, there is evidence that reasonably accurate quantification of perceived force exertion is achievable by healthy individuals. Hampton, Armstrong, Shah & Li (2014) evaluated accuracy of perceived force exertion during isometric finger contraction (calibrated by Borg scale[11] criteria) without visual or auditory feedback, in healthy and post-stroke individuals. Both groups demonstrated the capacity to differentiate distinct levels of perceived exertion, with the stroke group reporting greater perceived exertion for a given level of MVC. In healthy individuals, the overall trend was for a

[11] Borg scale: a well validated widely used research and clinical tool for quantifying perceived exertion, that correlates well with objective measures of workload and heart rate, and is used extensively in rehabilitation

subjective underestimation of force production when compared to the objectively measured magnitude for the percentage MVC requested.

Underestimation of the 10% MVC requested in our task was less problematic than overestimation, as there is evidence that contraction up to the 10% MVC level helps stabilize cortical and spinal excitability and reduce variability in the amplitude of single MEPs compared to the variability from MEPs obtained from muscles at rest (Darling, Wolf & Butler, 2006). Exceeding the 10% MVC threshold can result in an increase in MEP amplitudes due to the acknowledged relationship between increased voluntary activation of muscle[12] and greater MEP amplitudes (Di Lazzaro et al., 2004; Inghilleri, Berardelli, Cruccu & Manfredi, 1993; Kamen, 2004; Kojima et al., 2013). Analysis of our EMG_{RMS} and MEP amplitudes, in particular the direct comparison of 130% RMT and 130sp% RMT conditions, clearly indicated that participants performed the task as instructed (i.e., they contracted the FDI and APB muscles to grip the pen). Therefore, whilst it can be argued that lack of objective force verification introduced an additional uncontrolled variable into the study setting, the intention of the study was to detect change in M1/corticospinal activity in as naturalistic a setting as possible. When individuals draw/write, they do so with fluent procedural automaticity with no visual/auditory feedback. Whilst the provision of feedback to control force provides objective verification, it compromises ecological validity to a degree; for this study, the primary question benefitted from less interventionist control.

[12] via increased corticospinal pathway excitability

In summary

Previous research has reported inconsistent effects of brief duration motor activity on primary motor cortex excitability, either in the context of novel task learning paradigms, or in evaluation of plasticity-induction protocols utilising non-invasive brain stimulation tools such as TMS. In contrast, the effects of a routine, well-practiced, complex fine motor skill such as drawing, which is dependent on engagement of M1 and other cortical regions for execution, have received little formal investigation. Variability in TMS measures or responses to TMS-based protocols remains an incompletely understood problem, impacting on the use of TMS in all its applications, with antecedent factors theoretically important contributors to variability.

The present study used a naturalistic task (drawing) commonly performed by study participants prior to TMS testing, and demonstrated that performance of this type of task has no measurable effect on TMS measures up to 15 minutes post-task. To the extent that drawing can be considered a proxy for other routine activities of daily living, the findings suggest that the type of activities participants perform as part of their daily living are unlikely to significantly influence short-term TMS measures. Additionally, variability of the post-task MEP amplitudes were not significantly influenced by a task of this nature.

The unique features of this study were: (i) the use of a task with robust ecological validity, a crucial attribute enabling translation of research findings to clinical settings, and salient given emerging understanding that intracortical interactions operate in fundamentally different ways in the context of different behavioural, motor or cognitive demands (Reis et al., 2008); (ii) the formal assessment of

parameters of TMS excitation-inhibition for a well-practised task (at a level of procedural automaticity), as a proxy for an *activity of daily living*. Previous research into well-practised skills has typically evaluated tasks such as writing/drawing in the context of a control condition when studying neurological conditions such as hand dystonia (writer's cramp), or alternately sought to characterise the anatomical and functional differences underlying highly trained specialised activities such a piano playing by professional musicians in comparison to novices (Nordstrom & Butler, 2001; Ridding, Brouwer & Nordstrom, 2000). Common activities such as handwriting, typing, texting or object manipulation are, however, substantially more likely to be engaged in prior to a TMS study or therapeutic application than extensive instrument practice; and (iii) measurement of post-task effects – an under-investigated domain, but with emerging importance when considering paradoxical responses to plasticity protocols and the phenomenon of temporally delayed or indirect effects of plasticity induction.

Future research

Future research in this domain should include evaluation (pre-, intra- and post-task) of other common activities such as writing (with a text based task) or typing/keyboard use. Additionally, replication of this study, with inclusion of a plasticity-induction intervention (for example, a finger opposition sequence [FOS] learning task) at, say, 15 minutes post-drawing task completion, with measurement of discrete performance endpoints, compared to a matched group who undergo the same FOS learning task but without any preceding drawing (or similar) activity would enable a more definitive conclusion to be made about the effects of *complex overlearned* antecedent tasks (with both motor and cognitive elements) on plasticity induction interventions.

Conclusion

In conclusion, the present study aimed to address the gap in current knowledge regarding the magnitude and time course of effects of naturalistic tasks on motor cortex and corticospinal excitability. It was found that performance of a simple drawing task did not significantly change measures of corticospinal excitation-inhibition from the pre-task state. Thus for researchers testing participants who have engaged in writing activities prior to TMS application, the findings suggest that the antecedent motor activity will have negligible short-term impact.

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Table of Appendices

Appendix A: Ethics Requirements

A1: Ethics Approval Letter

A2: Information Sheet

A3: Consent Form

A4: Medical History and Handedness Questionnaires

A5: Advertisements for Participant Recruitment

Appendix A1: Ethics Approval Letter

Social Science Ethics Officer
Private Bag 01 Hobart
Tasmania 7001 Australia
Tel: (03) 6226 2763
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HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

UTAS

12 June 2014

Dr Michael Garry
Psychology
Private Bag 30

Sent via email

Dear Dr Garry

Re: APPROVAL FOR AMENDMENT TO CURRENT PROJECT
Ethics Ref: **H0009261 - Bilateral movement therapy in post-stroke hemiparesis**

- Change to investigators: addition of Honours students Ms Lillian Brinken and Ms Mona Thorpe, removal of Ms Monica Lovell.
- Change of task participants perform to a brief handwriting task.
- Narrowing of age range from 18-50 to 18-45.
- Revised Information Sheet and Consent Form to reflect changes to procedures and relocation of Psychology to the Faculty of Health.

We are pleased to advise that the Chair of the Tasmania Social Sciences Human Research Ethics Committee approved the Amendment to the above project on 11 June 2014.

Yours sincerely

Katherine Shaw
Executive Officer
Tasmania Social Sciences HREC

Appendix A2: Information Sheet

The effect of hand writing on cortical excitability

Information sheet for study participants

1. Invitation

You are invited to participate in a study investigating the effect of hand writing on the brain systems that control hand movements. The aim of the research is to improve our understanding of how well-learned tasks that are performed during our daily lives affect the neural systems that support the learning of novel tasks.

The study is being conducted by:

- Dr Mike Garry, School of Medicine (Psychology), University of Tasmania
- Ms Lillian Brinken (Honours student), School of Medicine (Psychology), University of Tasmania
- Ms Mona Thorpe (Honours student), School of Medicine (Psychology), University of Tasmania

This study is being conducted in partial fulfillment of Honours degrees for Lillian Brinken and Mona Thorpe under the supervision of Dr Mike Garry. The study will be take place in the Human Motor Control laboratory, Psychology Research Centre, University of Tasmania, (03) 2662 2204.

2. What is the purpose of this study?

The study is being conducted to improve our understanding of how the brain and nervous system are affected by the performance of common, everyday tasks. The specific focus of this study is whether, and how, handwriting tasks influence the parts of the brain and nervous system that control movement.

The findings from this study will help to improve understanding of how the brain and nervous system control movements. This knowledge will help with the development and refinement of rehabilitation therapies for people that have suffered brain injuries such as stroke.

3. Why have I been invited to participate?

As you are between 18 and 45 years of age, are right-handed, and have normal or corrected-to-normal vision you have been invited to participate in this research. We want to emphasise that your participation is voluntary and that you are free to withdraw at any time.

The technique of transcranial magnetic stimulation (TMS) used in this study is very safe, but there are certain conditions that will exclude some people from participating. You will be

asked to complete a medical screening questionnaire to ensure that you are free of any exclusionary criteria.

Exclusion criteria include:

- epilepsy, or a family history of epilepsy
- history of unexplained seizures (fits)
- serious head injury (e.g., concussion) requiring hospitalisation within the last three years
- implanted electronic devices such as pacemakers
- metal implants or metal fragments in the head (excluding dental work)
- history of migraines
- pregnancy

> Certain medications (for example some types of anti-depressant medications) can influence how the brain responds to sensory stimulation and voluntary movements. Therefore, we ask that you inform the experimenter if you are taking any medication prior to participating in the study.

4. What will I be asked to do?

This study will involve you completing two (2) separate testing sessions, each lasting approximately 90 minutes, at least seven (7) days apart. These will be scheduled at times that are convenient for you. Prior to the first session you will be asked to complete a short, questionnaire to collect demographic information (age, sex, etc.), assess handedness and screen for exclusion criteria for transcranial magnetic stimulation (TMS). If you are free of all exclusion criteria you continue to the main part of the study.

At the beginning of each session sticky recording electrodes will be placed on the skin over two muscles of your right hand: one muscle that moves your index finger, and one muscle that moves your thumb. To ensure the best possible recording of the activity of these muscles, the skin will be prepared by scrubbing it with a mildly abrasive paste and then cleaning it with an alcohol wipe. If there is hair on the skin a small area will be shaved using a disposable razor. This procedure may produce some minor irritation of the skin (e.g., redness). The adhesives used on the electrodes are hypoallergenic. Wires will then be connected to the electrodes so a recording device (EMG system) can record muscle activity during the experiment.

The technique of transcranial magnetic stimulation (TMS) will be used to stimulate the area of the brain that controls muscles of the right hand. TMS is a safe, painless technique used to measure changes in the activity of the brain during the study. Electromagnetic 'pulses' will be delivered through a coil held against your scalp by the investigator. To ensure the coil is always positioned in the same place, a felt-tip pen will be used to mark the location on your scalp. This mark will be removed at the end of the session using an alcohol wipe. When a TMS pulse is delivered you will hear 'click' sound from the coil and muscles of the hand/arm will 'twitch'. You may also feel a 'tap' sensation on your scalp and muscles around the eye may twitch, causing the eye to blink. This may feel a bit strange but it is not painful.

TMS will be used to measure brain activity at four time points ('blocks') during the study. Each of these blocks of TMS stimulation will take approximately nine (9) minutes to

complete, and there will be approximately six minutes between blocks. Approximately 100 TMS pulses will be delivered in each block. For the majority of the block you will be asked to sit quietly with your hand muscles relaxed, but for approximately one minute of each block you will be asked to lightly grip a pen held between the index finger and thumb of your right hand.

During the interval between the second and third TMS block you will be asked to perform simple handwriting task. This task will differ in the two sessions. In one session you will be asked to copy a 120 word passage of text onto a piece of paper. In the other session you will be asked to repeatedly draw a set of three geometric symbols: a circle (○), cross (×), and a tick (✓). In total you will draw this set of symbols 200 times. Following the handwriting task, the remaining two blocks of TMS will be given.

After the final TMS block, the electrodes will be removed from your hand and you will be free to leave.

5. Are there any possible benefits from participation in this study?

Your involvement in this study will aid in the understanding of the brain and nervous system's role in the control of movement. The findings from the study will contribute to the development of techniques to improve recovery of function following brain injury, such as stroke.

First-year psychology students will receive 3 hours course credit following completion of both sessions (i.e., 1.5 hours for each session). If you are not a first year student, or have already received full participation credit, you be entered into a draw to receive one of two $50 Coles-Myer gift vouchers.

6. Are there any possible risks from participation in this study?

There are few risks associated with the procedures used in this study. The TMS pulse may cause muscles of the scalp to 'twitch' (e.g., can cause the eye to blink). This may feel 'odd', but is not painful. On rare occasions TMS can cause a 'muscle tension' type headache.

TMS requires self-adhesive electrodes to be place on the skin. The skin will need to be prepared prior to application of these electrodes. This will involve scrubbing the skin with a mildly abrasive paste and shaving the skin using a disposable razor to remove any hair. These may cause some mild skin irritation and redness.

Some people experience 'vasovagal syncope', or fainting, in response to certain 'trigger' stimuli. Common triggers for sensitive individuals include health-related procedures, such as needles or the sight of blood, and stress and anxiety. For a small percentage of people, TMS can trigger a fainting reaction. If you have experienced fainting previously, please let us know.

7. What if I change my mind during or after the study?

It is important that you understand that your participation in this study is completely voluntary and you are free to withdraw at any time without prejudice. If you decide not to

participate you may do so without providing an explanation. Prior to study participation you will be asked to sign a Statement of Informed Consent to indicate your full understanding of the purpose and requirements of your participation. However, if you find that you are becoming distressed, we will arrange for you to see a University counsellor at no expense to you. Should you choose to withdraw from the study, any information provided during your participation will, if possible be excluded from the study.

8. What will happen to the information when this study is over?

After this study has been completed, all data will be kept for five years. Electronic documents will be stored on a password protected computer in the Human Movement and Neuroscience Laboratory at the University of Tasmania, Hobart Campus. All other documents will be stored in locked filing cabinets on the Hobart Campus. All information will be treated in a confidential manner, and your name will not be used in any publication arising out of the research. This data can only be accessed by the Chief Investigator and Student researcher. After a five year duration the data will be destroyed by deletion of electronic documents and shredding of other documents.

9. How will the results of the study be published?

The results of this study will be disseminated in a research thesis, as well as in a presentation to fellow Honours students and their supervisors. The study results will also be submitted for publication in a peer-reviewed, neuroscience research journal. Participants will not be identifiable in the publication of results.

10. What if I have questions about this study?

If you would like to discuss any aspect of this study please feel free to contact either Mike Garry on (03) 6226 2204, Lillian Brinken (lbrinken@postoffice.utas.edu.au), or Mona Thorpe (mthorpe0@postoffice.utas.edu.au). Any of us would be happy to discuss any aspect of the research with you. Once we have analyzed the information we will be mailing / emailing you a summary of our findings. You are welcome to contact us at that time to discuss any issue relating to the research study.

This study has been approved by the Tasmanian Social Sciences Human Research Ethics Committee. If you have concerns or complaints about the conduct of this study, please contact the Executive Officer of the HREC (Tasmania) Network on +61 3 6226 7479 or email human.ethics@utas.edu.au. The Executive Officer is the person nominated to receive complaints from research participants. Please quote ethics reference number [H0009261].

Thank you for taking the time to consider this study. If you wish to take part in it, please sign the attached consent form. This information sheet is for you to keep.

Appendix A3: Consent Form

The effect of hand writing on cortical excitability

This consent form is for research participants.

1. I agree to take part in the research study named above.
2. I have read and understood the Information Sheet for this study.
3. The nature and possible effects of the study have been explained to me.
4. I understand that the study involves two sessions of approximately 90 minutes each, at least seven days apart. In each session, sticky electrodes will be placed on my right hand to allow recording of muscle activity, and transcranial magnetic stimulation will be used to measure brain activity. I will perform a short handwriting task (approximately five minutes) in each session.
5. I understand that participation involves the risk(s) that skin preparation for muscle recording may cause mild discomfort and that transcranial magnetic stimulation will produce a click sound and muscle twitches of the face and hand. I will complete a medical screening questionnaire to ensure I am free of exclusion criteria for transcranial magnetic stimulation.
6. I understand that all research data will be securely stored on the University of Tasmania, Sandy Campus premises for five years from the publication of the study results, and will then be destroyed unless I give permission for my data to be stored in an archive.

   I agree to have my study data archived.

   Yes ☐ No ☐
7. Any questions that I have asked have been answered to my satisfaction.
8. I understand that the researcher(s) will maintain confidentiality and that any information I supply to the researcher(s) will be used only for the purposes of the research.
9. I understand that the results of the study will be published so that I cannot be identified as a participant.
10. I understand that my participation is voluntary and that I may withdraw at any time, without prejudice, if I wish.

    If I so wish, I may request that any data I have supplied be withdrawn from the research until August 31, 2014 after which the data will be included in the Honours theses of Mona Thorpe and Lillian Brinken.

Participant's name: _____

Participant's signature: _____Date: _____

Statement by Investigator

☐ I have explained the project and the implications of participation in it to this volunteer and I believe that the consent is informed and that he/she understands the implications of participation.

If the Investigator has not had an opportunity to talk to participants prior to them participating, the following must be ticked.

☐ The participant has received the Information Sheet where my details have been provided so participants have had the opportunity to contact me prior to consenting to participate in this project.

Investigator's name: _____

Investigator's signature: _____

Date: _____

Appendix A4: Medical History and Handedness Questionnaires

Medical History and Handedness

Participant Code..... Age..... Sex: M / F

Exclusion criteria

Do any of the following apply to you?

- epilepsy, or a family history of epilepsy yes no
- history of unexplained seizures (fits) yes no
- serious head injury (e.g., concussion) that required hospitalisation within the last three years yes no
- implanted electronic devices such as pacemakers yes no
- metal implants or metal fragments in the head (excluding dental work) yes no
- history of migraines yes no
- currently pregnant or could be pregnant yes no

Medical History

Are you currently suffering from anxiety or depression?.....

Do you have a heart condition or any other serious physical condition?

.....

Are you currently taking any prescription medication? If so, what medication?

.....

Have in the past taken any medications for psychological condition(s)? If so, what medications?

.....

Have you ever had or are you now suffering from any of the following (please circle):

Stroke	Yes	No
High Blood Pressure > 140 / 90	Yes	No
Diabetes	Yes	No
Arthritis	Yes	No
Fits or convulsions	Yes	No
Epilepsy	Yes	No
Giddiness	Yes	No
Concussion	Yes	No
Severe Head Injury	Yes	No
Loss of Consciousness	Yes	No

Handedness

For each of the activities below, please tell us:

1. Which hand do you prefer for that activity?
2. Do you *ever* use the other hand for the activity?

	Preferred hand?		Ever use other hand?	
Writing	L	R	Y	N
Drawing	L	R	Y	N
Throwing	L	R	Y	N
Using scissors	L	R	Y	N
Using a toothbrush	L	R	Y	N
Using a knife (without fork)	L	R	Y	N
Using a spoon	L	R	Y	N
Using a broom (upper hand)	L	R	Y	N
Striking a match	L	R	Y	N
Opening a box (lid)	L	R	Y	N

Do you ever confuse left and right?……..

How many people in your immediate family are left handed?……

Thank you.

Appendix A5: Advertisements for Participant Recruitment

Faculty of Health (Psychology)

UTAS

Research Participation

We are currently seeking **right handed research volunteers**, **aged 18-45 yrs** to participate in a study investigating how hand writing affects the brain and nervous system.

The study will help us understand how the brain and nervous system contribute to the control of well-learned skills that we perform daily, such as handwriting, The findings from this study will help to improve the development and refinement of therapies to assist the recovery of movement after brain injury such as stroke.

The research will involve two sessions of approximately 90 minutes each, at least seven days apart. If you are a first year Psychology student, you will receive course credit for the total time you were involved with the experiment. If you are a non-psychology student or already have obtained full course credit you will be entered into a draw for one of two $50 Coles-Myer gift vouchers.

If you are interested in taking part in this research project, or would like more information, please contact Lillian Brinken (lbrinken@utas.edu.au) or Mona Thorpe (mthorpe0@utas.edu.au) for further information.

Do you love science?

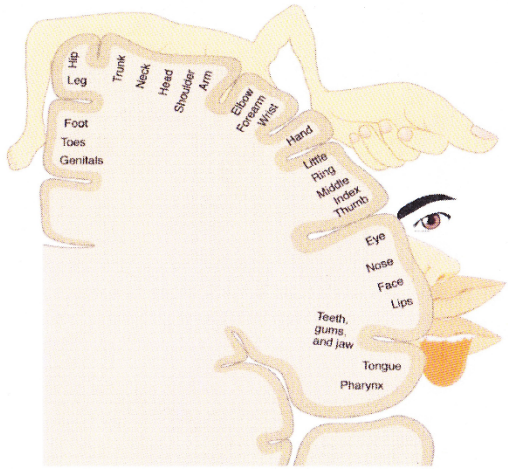
How much? Enough to help science if science needed you? Here is your chance to show how much you love science by participating in an exciting, non-invasive study into Neuroplasticity and every-day tasks. Eligible participants need to be right-handed and aged between 18-45. Course credit available for first year Psychology students; other participants go into a draw to win one of two $50 Coles-Myer gift vouchers.

Email Lily, lbrinken@utas.edu.au or Mona, mona@utas.edu.au for more information.

Study: The effect of handwriting on cortical excitability

Chief Investigator: Dr Mike Garry

Ethics Approval no. HOOO9261



Study participation requires attendance at the Motor Control Lab at the Sandy Bay Campus of UTAS, for two 90 minute sessions, approximately a week apart. Launceston campus students are very welcome to participate if able to commit to attendance at both sessions.

Appendix B: Experimental Drawing Task

The effect of handwriting on cortical excitability study

Motor Control Lab, Division of Psychology, School of Medicine, UTAS

Supervisor: Dr Mike Garry; Honours students: Mona Thorpe, Lily Brinken

For this drawing task, please copy the 3 symbols depicted, in order (circle, cross, tick), into each box of the grid, using a comfortable handwriting speed. This task does not have a time limit so you are free to adjust your writing speed to minimise hand muscle fatigue.

o✕✓									

Appendix C: Statistical analyses

C1: Background muscle EMG_{RMS} data analysis

C2: MEP amplitude data analysis

Appendix C1: Background muscle EMG_{RMS} data analysis

Table **1**. Marginal means for background EMG_{rms} activity for FDI and APB muscles

Table **1.1** TMS type

Muscle	TMS Type	Mean (mV)	Standard Error	95% Confidence Interval
APB	TS	.015	0.004	0.007 - 0.023
	CS	.015	0.004	0.006 - 0.024
FDI	TS	.010	0.002	0.007 - 0.014
	CS	.010	0.001	0.006 - 0.013

Table **1.2** Time

Muscle	Time	Mean (mV)	Standard Error	95% Confidence Interval
APB	B1	.014	0.004	0.006 - 0.022
	B2	.015	0.005	0.005 - 0.026
	Post0	.015	0.004	0.006 - 0.024
	Post15	.016	0.004	0.006 - 0.026
FDI	B1	.009	0.002	0.005 - 0.014
	B2	.010	0.002	0.006 - 0.014
	Post0	.011	0.001	0.007 - 0.014
	Post15	.010	0.002	0.007 - 0.014

Table **1.3** Intensity

Muscle	Intensity	Mean (mV)	Standard Error	95% Confidence Interval
APB	120	.008	0.003	0.002 - 0.013
	130	.008	0.004	-0.001 - 0.017
	140	.007	0.002	0.001 - 0.013
	130sp	.037	0.013	0.007 - 0.068
FDI	120	.007	0.002	0.003 - 0.011
	130	.007	0.002	0.003 - 0.011
	140	.007	0.002	0.003 - 0.011
	130sp	.018	0.003	0.012 - 0.025

Appendix C2: MEP amplitude data analysis

Table **1**. MEP amplitude means, SE and CI for FDI and APB muscles

Table **1.1** Main effect of *TMS type* on mean MEP amplitude for FDI and APB muscles.

Muscle	TMS type	Mean (mV)	Standard Error	95% Confidence Interval
FDI	TS	3.770	0.683	2.155 - 5.384
	CS	1.832	0.313	1.092 - 2.572
APB	TS	2.670	0.555	1.357 - 3.984
	CS	1.810	0.659	0.253 - 3.368

Table **1.2** Main effect of *time* (across TMS types and intensity) on mean MEP amplitude for FDI and APB muscles.

Muscle	Time	Mean (mV)	Std. Error	95% Confidence Interval
FDI	B1	2.614	0.399	1.672 - 3.557
	B2	2.872	0.376	1.983 - 3.762
	Post0	2.740	0.485	1.593 - 3.888
	Post15	2.977	0.495	1.805 - 4.148
APB	B1	2.060	0.574	0.703 - 3.418
	B2	2.216	0.611	0.771 - 3.662
	Post0	2.142	0.584	0.760 - 3.524
	Post15	2.542	0.640	1.029 - 4.054

Table **1.3** Main effect of *intensity* (across TMS type and time) on mean MEP amplitude for FDI and APB muscles.

Muscle	Intensity	Mean (mV)	Std. Error	95% Confidence Interval
FDI	120	1.319	0.267	0.688 - 1.951
	130	2.000	0.367	1.132 - 2.869
	140	3.154	0.577	1.791 - 4.518
	130sp	4.729	0.656	3.177 - 6.281
APB	120	1.220	0.430	0.202 - 2.237
	130	1.743	0.507	0.544 - 2.942
	140	2.301	0.547	1.008 - 3.595
	130sp	3.696	1.035	1.250 - 6.143

Table **1.4**. FDI muscle: Effect of *time* on *single-pulse (TS)* (upper panel) and *paired-pulse (CS)* (lower panel) TMS-elicited MEP mean amplitudes (mV), ± SE, and CI. The main effect of *time* was not significant for either TMS_{TS} or TMS_{CS}.

Muscle (TMS type)	Time	MEP Mean (mV)	Std. Error	95% Confidence Interval
FDI (TS)	B1	3.470	0.667	1.890 – 5.044
	B2	3.951	0.650	2.414 – 5.488
	Post0	3.688	0.775	1.856 – 5.520
	Post15	3.972	0.719	2.272 – 5.673
FDI (CS)	B1	1.762	0.265	1.135 - 2.389
	B2	1.794	0.358	0.948 – 2.639
	Post0	1.793	0.329	1.014 – 2.571
	Post15	1.981	0.440	0.940 - 3.022

Table **2**. APB muscle: Pairwise comparisons of MEP amplitude across time points, for each MEP type (TS, CS), with Bonferroni correction for multiple means, significance level .05

MEP type	time (I)	time (J)	Mean Difference (I-J)	Std. Error	Sig.	95% CI for Diff.
TS	B1	B2	-.237	0.070	.067	-0.490 - 0.016
		Post0	-.039	0.160	1.000	-0.621 - 0.543
		Post15	-.603	0.203	.125	-1.343 - 0.136
	B2	B1	.237	0.070	.067	-0.016 - 0.490
		Post0	.198	0.185	1.000	-0.475 - 0.872
		Post15	-.366	0.209	.737	-1.125 - 0.393
	Post0	B1	.039	0.160	1.000	-0.543 - 0.621
		B2	-.198	0.185	1.000	-0.872 - 0.475
		Post15	-.564	0.201	.159	-1.297 - 0.168
	Post15	B1	.603	0.203	.125	-0.136 - 1.343
		B2	.366	0.209	.737	-0.393 - 1.125
		Post0	.564	0.201	.159	-0.168 - 1.297
CS	B1	B2	-.074	0.084	1.000	-0.379 - 0.231
		Post0	-.124	0.213	1.000	-0.898 - 0.650
		Post15	-.359	0.182	.537	-1.021 - 0.303
	B2	B1	.074	0.084	1.000	-0.231 - 0.379
		Post0	-.050	0.176	1.000	-0.689 - 0.589
		Post15	-.285	0.148	.569	-0.822 - 0.252
	Post0	B1	.124	0.213	1.000	-0.650 - 0.898
		B2	.050	0.176	1.000	-0.589 - 0.689
		Post15	-.235	0.157	1.000	-0.804 - 0.334
	Post15	B1	.359	0.182	.537	-0.303 - 1.021
		B2	.285	0.148	.569	-0.252 - 0.822
		Post0	.235	0.157	1.000	-0.334 - 0.804